



UNITED STATES NAVY

MEDICAL NEWS LETTER

Editor - Captain F. W. Farrar, MC, USN

Vol. 13

Friday, 8 April 1949

No. 7

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Neomycin, a New Antibiotic Active Against Streptomycin-Resistant Bacteria, Including Tuberculosis Organisms: In the 25 March 1949 issue of *Science*, Selman A. Waksman and Hubert A. Lechevalier of the Department of Microbiology, New Jersey Agricultural Experiment Station, Rutgers University, report the discovery of an antibiotic which they have designated, neomycin.

The organism producing neomycin was isolated from the soil and is somewhat related to an organism isolated from the soil in 1915 by Waksman and Curtis and designated as Actinomyces fradii (now listed in Bergey's Manual as Streptomyces fradiae). When the newly isolated organism was grown in various media containing a source of nitrogen (peptone, meat extract), a carbohydrate (glucose), and salt (NaCl), it was found to produce neomycin under both stationary and submerged conditions of culture. The antibiotic can be removed easily from the culture medium and concentrated by the methods of adsorption and elution which have been developed for the isolation of streptothricin and streptomycin from their respective culture media.

Neomycin is a basic compound, most active at an alkaline reaction. It is soluble in water, insoluble in organic solvents, thermostable, and active against numerous Gram-positive and Gram-negative bacteria, especially mycobacteria, but not against fungi. The antibiotic spectrum of the crude neomycin is quite distinct from that of streptomycin, and of streptothricin as shown in the table below.

ANTIBIOTIC SPECTRUM OF CRUDE NEOMYCIN, AS COMPARED TO THAT OF CRUDE STREPTOMYCIN AND STREPTOTHTRICIN

Test organism	Dilution units per gm required to inhibit growth of test organisms		
	Neomycin	Streptomycin*	Streptothricin*
<i>B. subtilis</i>	150,000-750,000	125,000	125,000
<i>B. mycoides</i>	20,000-150,000	20,000	1,000
<i>B. cereus</i>	20,000-60,000	30,000	1,000
<i>S. aureus</i>	100,000-250,000	15,000	50,000
<i>S. lutea</i>	10,000	100,000	37,500
<i>E. coli</i> SS†	25,000	25,000	25,000
<i>E. coli</i> RS‡	20,000	0	Active§
<i>Ps. aeruginosa</i>	2,500	1,000	1,000
<i>Pr. vulgaris</i>	25,000	10,000	12,500
Bodenheimer's organism	15,000	0	Active§
<i>Serratia marcescens</i>	20,000	25,000	1,200
<i>Mycobacterium</i> 607	80,000-250,000	Active§	Active§
<i>Mycobacterium</i> 607RS	50,000-150,000	Inactive	Active§
<i>Trichophyton mentagrophytes</i>	< 300	< 300	Active§
<i>Candida albicans</i>	< 300	< 300	Active§

* From Shatz, Bugie and Waksman (8).

† SS = streptomycin-sensitive.

‡ RS = streptomycin-resistant.

§ Active = activity established in other reports.

Neomycin preparations were found to possess several desirable properties which fully justified a more detailed study; (1) similar activity against both streptomycin-sensitive and streptomycin-resistant bacteria; (2) considerable activity (in some cases greater activity than streptomycin) against various forms of Mycobacterium tuberculosis and other mycobacteria; (3) limited, or no toxicity to animals; (4) activity against various bacteria *in vivo*,

including Gram-positive and Gram-negative organisms and against both streptomycin-sensitive and streptomycin-resistant organisms; (5) lack of resistance against neomycin among the organisms sensitive to it, or only limited development of such resistance.

When a 20-hour-old agar culture of Escherichia coli was suspended in water and plated out in nutrient agar containing 5 units or 25 units of neomycin per ml., no colonies of E. coli developed out of 22 billion cells, after 9 days' incubation at 28° C. Similar concentrations of streptomycin would usually allow the development of a dozen or more bacterial colonies per plate. When pieces of agar were removed from the plates containing neomycin and inoculated into sterile agar plates, only the 5 units per ml. plate gave growth from some of the pieces, but the 10 units per ml. and the 25 units per ml. plates gave no growth at all, thus pointing to the high bactericidal properties of neomycin. When plates containing different concentrations of this antibiotic were streaked with streptomycin-sensitive, -resistant, and -dependent strains of E. coli, the first two were sensitive alike to neomycin, and the last did not make any growth at all. This established further the difference in the biological and chemical nature of neomycin and streptomycin.

Broth or agar cultures containing sufficient neomycin to inhibit the growth of various bacteria were incubated for considerable periods of time. No further bacterial development occurred beyond a certain initial inhibiting concentration, thus pointing to the stability of neomycin, as contrasted to that of aureomycin, and to the lack of resistance developed among the sensitive bacteria, as contrasted to streptomycin and especially to grisein.

Because neomycin has not yet been obtained in crystalline form, very little can be said of its chemical nature. Preliminary results, however, point to its being distinctly different chemically from streptothricin and from streptomycin. More detailed studies will be published later.

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Foreign Bodies in the Air and Food Passages: The material presented is based on the author's consecutive series of 250 cases of foreign body (1941-1946) at the Chevalier Jackson Bronchoscopic Clinic. Only cases in which a foreign body was removed by peroral endoscopy are included in this group; cases of suspected foreign body, in which the presence of a foreign body was ultimately disproven, and cases in which the foreign body was allowed to pass spontaneously through the gastro-intestinal tract have been omitted.

In this series, as in those reported by other authors, the age factor appeared to be closely related to the incidence of the various common types of foreign body. Coins, safety pins and vegetal foreign bodies are seen almost without exception in infants and young children, whereas bones and meat occur

almost exclusively in patients of the older age group. The incidence of all types of foreign body in the second decade is extremely low.

In 70 patients (28 percent of the entire series) bones were the foreign body. More than half of these were chicken bones, and those of flat triangular shape predominated, although nearly one third were of the double-pointed or spicular variety (fragments of rib or long bones).

Delays in diagnosis were infrequent in this group of cases, because the symptoms were of sudden onset, continuous and sufficiently severe to demand prompt attention. The patients, almost without exception, consulted a physician immediately or within a few hours, and subsequent delays can in nearly every case be attributed to erroneous impressions of the first physician consulted. There were 16 cases in which the advice of the first physician consulted resulted in delay. Nine of these, patients had been told, following examination by the indirect method or by palpation with the finger, that there was probably nothing there, that the foreign body had gone down, or that the throat had been merely irritated by temporary lodgment of the foreign body. These patients had been dismissed with gargles or lozenges, without the suggestion that an x-ray examination be made. In several instances the physician had suggested swallowing dry bread or other coarse material in an effort to dislodge the bone, and in three cases an attempt had been made to push the bone down with a rubber tube or bougie.

Roentgen examination of the neck, the swallowing function and the esophagus was carried out in all patients except for the few in whom a bone could be seen in the hypopharynx on mirror examination and its relations well determined. Nearly all of the chicken bones were sufficiently opaque to be visible in the lateral neck film, but in a few instances, films made after administration of barium sulfate suspension were more conclusive. The fish bones, though less opaque, could also as a rule be identified in the lateral neck film; adherence of barium added further evidence in several of these patients.

There were only five cases in which a bone had passed beyond the cervical esophagus; in all of these, the foreign body had lodged at the level of the aortic arch. In three of these, the x-ray examination was positive, in one negative (a slender spicule of pork bone), and in one inconclusive.

Removal was accomplished without general anesthesia in all 70 of the bone cases. The adolescent sized laryngoscope was used for most of the patients with bone in the hypopharynx. An esophageal speculum of adult size was employed in several of the patients with bone in the cervical esophagus, but usually an esophagoscope of large caliber was preferred. The bones most difficult to find were those at or just below the cricopharyngeal level, particularly in patients in whom edema and ulceration had occurred as a result of several days' delay.

The mechanical problems were those of dealing with multipointed objects. Complications were infrequent in this group of patients.

In 40 patients (16 percent of the entire series), coins were the foreign body. All of the patients were children; only five were over five years old. In six patients there were respiratory symptoms in addition to the usual complaints of gagging and regurgitation on attempted feeding. In four of these patients the foreign body was discovered only when x-ray films were taken because of persistent wheezing, croupy cough and mild dyspnea; in one case these symptoms had been present for over a month, having been attributed to enlargement of the thymus gland. Significant delays in diagnosis were not frequent, however. Twenty-one of the patients were seen in less than 48 hours, and for only eight was the interval greater than a week. In all except four patients, the coin was in the cervical esophagus, lying in the usual coronal plane. In two of the four patients in whom a coin had passed on into the thoracic esophagus, arrest had occurred at the level of the aortic arch. In one patient, a quarter had lodged in the lower thoracic esophagus of a nine-year-old boy; in the fourth patient, a penny was found above the stenotic esophagogastric junction of a two-year-old girl with congenitally short esophagus. In all of these cases removal was accomplished without complication and without use of any anesthesia, general or local. The esophagoscope was used in only five patients, including the four of coins in the thoracic esophagus. In 30 patients the esophageal speculum, small size, was used, and in five young infants, the infant sized laryngoscope was found to provide adequate visualization through the cricopharyngeal orifice.

The group with safety pins comprises 33 patients (13.2 percent of the entire series). Three of the safety pins were in the bronchus (two closed, one open), 17 were in the esophagus, and 13 were found in the hypopharynx. In all except one of these patients the presence of a foreign body had been recognized promptly. In 19 patients, an episode of choking and gagging had been heard or observed by the parents or others; this was followed in some cases by a brief interval which was essentially asymptomatic except that in patients in whom feedings were attempted, reluctance to eat and regurgitation were observed. Eight of the children were old enough to report to their parents the fact that they had swallowed a pin. In three instances, refusal of a child to eat led to roentgen examination which revealed the presence of an open safety pin. The case in which the foreign body was overlooked was one of a safety pin in the bronchus; the patient was a child of two years who had been treated medically for about six weeks (first for a common cold, then for pneumonia). Roentgen examination at the end of this time had shown a small open safety pin, apparently in the left bronchus. In nine of the patients the pin was shown by x-ray to be lodged at the cervical level of the esophagus, point uppermost. In five patients, the open pin had been arrested at the level of the aortic arch; in two of these patients the pin was lying point down, with spring end uppermost. In the remaining three patients, the open safety pin was in the lower thoracic esophagus. In none of the patients did the

preliminary examination suggest spontaneous perforation. All of the safety pins in the hypopharynx were removed by the dangling method except in two cases in which the point was easily seen and grasped. In all of the esophageal cases, except the two in which the pin was lodged spring end uppermost, removal was accomplished by endogastric version under biplane fluoroscopic guidance, using a ring rotation forceps. In one patient in whom the pin did not pass easily through into the stomach, version was accomplished in the lower thoracic esophagus. In four patients, a forceps alone, without esophagoscope, was used. This forceps is an ordinary ring rotation forceps of medium weight, provided with a retractable rubber sheath which protects the forceps blades during introduction through the cricopharyngeal orifice. After it is advanced to the level of the spring end of the pin, the sheath is retracted, a grasp obtained through the spring with fluoroscopic guidance, and the pin then carried into the stomach, allowed to rotate, and brought out with the point trailing. Passage of the forceps through the hiatal opening is facilitated if the forceps is slightly bowed before introduction, so that when being advanced down the esophagus the convexity of its curve is posterior. The chief application of this modified forceps appears to be in cases (particularly in young infants) in which dyspnea due to compression of the trachea from behind occurs on introduction of the esophagoscope.

In 23 patients meat was the foreign body; 18 of the patients were over the age of 40 years. In all of these patients except one, the foreign body was below the cricopharyngeal level; this patient was a 73-year-old female who subsequently proved to have a carcinoma just below the cricopharyngeal level. In seven of the patients, some variety of obstructive esophageal lesion appeared responsible for the lodgment of a bolus which would probably have passed through a normal esophagus. Four of these patients had lye strictures of long duration; in two patients the stenosis was in association with a congenitally short esophagus, and in one patient the cause of the stenosis was undetermined. These patients presented no particular mechanical problem; in most cases piecemeal removal was accomplished through the esophagoscope by use of the fenestrated meat forceps.

In 25 patients (10 percent of the entire series) there was a vegetal foreign body. All of these patients except one were children, 19 of the 25 being under three years; two were less than one year of age. The foreign body was in the trachea in 4 patients, in the right main stem or lower lobe bronchus, in 17; there were only 4 patients in whom a vegetal foreign body occurred in the left bronchus. In 22 (88 percent) of these 25 patients, there had been a definite or strongly suggestive history of foreign body. An initial episode of choking and cough, with or without dyspnea or cyanosis, had been observed by the parents or others. In the ensuing period, cough, not always frequent or severe, was noted, but it appeared that persistent wheeze was a somewhat more constant and disturbing symptom in this interval. Appreciable degrees of dyspnea and cyanosis occurred in only four of the patients during the interval period, although these symptoms were noted initially in about half of the patients. In three cases

in which the initial episode was not observed, a physician was consulted because of cough and wheezing which failed to subside. Delays in diagnosis were more frequent than would be expected in view of the obvious symptoms and the circumstances in which they had their onset. Although 6 of the patients were seen in the Bronchoscopic Clinic within the first 24 hours and 4 within the second 24 hours, the interval in 12 patients was greater than one week. Five were first seen during the second week, 4 during the third, and one during the fourth. In one case (peanut) the interval was about 5 weeks, and in another (broccoli leaf) about 2 and 1/2 months. In 6 of the illnesses of more than one week's duration, the parents were responsible for avoidable delay through failure to consult a doctor. However, the first physician seen was chiefly responsible for delay in an equal number of cases through failure to suggest an x ray examination, or through failure to realize that a roentgen examination that is allegedly negative does not necessarily rule out the possibility of a nonopaque foreign body. For several patients medical treatment (for pneumonia, tonsillitis or asthma) had been prescribed, and for two patients the parents had been advised to await the development of more severe symptoms before doing anything further. The findings on physical examination of the chest were abnormal in all of the patients; this fact is of considerable importance in view of the fact that the initial roentgen examination, including fluoroscopy, was negative in two of the four patients with a foreign body in the trachea and in three of the 21 patients with a foreign body in the bronchus. Wheeze was the most obvious and frequent finding in all of the patients except those in whom atelectasis had occurred. In all of the tracheal cases and in most of the bronchial cases it was easily heard at the open mouth. In the bronchial cases in which wheezing was heard only on auscultation, it appeared at times that the wheeze was heard with almost equal intensity on the two sides. Bronchoscopic removal was accomplished in all of the patients. In 20, removal was apparently complete at the time of the first bronchoscopy; in the other five patients, subsequent bronchoscopy, carried out after an interval of two or three days because of persistent abnormal physical or x-ray findings, showed one or more additional fragments, presumably the result of fragmentation at the time of the initial grasp. In two of the five patients in whom additional fragments were removed, the interval roentgenogram had been reported negative; it appears that physical examination is equal in importance to the roentgenographic findings in determining whether removal has been complete, for the two patients mentioned were afebrile, essentially asymptomatic, and might have been discharged had not auscultation suggested the possibility of residual foreign body. One or more additional bronchoscopies were done for purposes of aspiration with the idea of facilitating re-aeration in the patients in whom atelectasis had been present. Tracheotomy following bronchoscopy was required in one case (peanut shell in the trachea of a 13-month-old boy), and had been performed prior to admission to the Bronchoscopic Clinic in one case (bean in the right main bronchus of an eight-month-old girl). In the latter case, the tracheotomy had failed to relieve the dyspnea, apparently because the obstruction was below the level of the tracheotomy. This infant appeared moribund when first seen, with temperature of 106.6° F., grayish pallor, barely audible heart beat, feeble respiratory effort and weak convulsive movements. Oxygen was insufflated through the bronchoscope

as the bean was grasped and withdrawn, and the bronchoscope again introduced for aspiration and further insufflation. Although oxygen was required for the first day, there was no dyspnea or cyanosis thereafter, and the temperature subsided within 24 hours. Decannulation was possible during the third week, and reaeration of the right lung was complete by the twentieth day. The average duration of hospitalization for the patients with vegetal foreign body was 10.3 days; patients were discharged only after careful and repeated auscultation showed satisfactory aeration of both lungs, with normal temperature for at least three or four days and, in all except four cases, roentgen findings which were essentially negative. In these four cases, the residual densities seen on x-ray study were minimal and apparently resolving, although complete resolution, as determined by comparative roentgenograms made following discharge from the hospital, required considerable time (60 days in one case; 86 days in another).

In 5 patients dentures were the foreign body; In two of these patients, the accident had occurred during sleep; one of the patients had swallowed his denture while celebrating his discharge from the Service, and another, an epileptic, during one of her seizures. Only one of the dentures had been arrested in the cervical esophagus; three were at the level of the aortic arch, and one in the lower thoracic esophagus. As is expected from the fact that many dentures are constructed of materials which are nonopaque, there was difficulty in determining from the x-ray film the size, shape, and relations of the foreign body. In one patient two long and unusually sharp projecting roots of peg teeth were not apparent on the films, and the patient, though questioned before removal was attempted, had not been aware of their presence. Removal was accomplished from all of these patients. The mechanical problems, aside from those relating to the size of the object, were in protecting the projecting parts during withdrawal. There were no complications in this group of patients; the average time of hospitalization was 2.6 days.

There were 7 patients with tracheal foreign body including four of vegetal matter, two of wooden beads, and one a rather large ovoid pebble. Jackson has called attention to the difficulties of roentgen diagnosis in foreign bodies in the trachea, and in three of the present group of cases (one peanut, one grain of corn, one wooden bead), the roentgen examination, including fluoroscopy, was reported negative. In three of the patients bilateral obstructive emphysema, the expected finding in foreign body of appreciable size in the trachea, was observed, and in the patient with the pebble, the foreign body was of sufficient density to be visible in the tracheal lumen on the lateral film. All of these patients had an obvious wheeze at the open mouth. In one of the bead cases, that of a three-year-old boy, the wheeze had been continuously present for 17 months. Tonsillectomy had been advised and performed shortly after the onset of the wheezing, and subsequently x-ray treatments were administered for a supposedly enlarged thymus. Allergy

studies and treatment had occupied a period of several months before the possibility of foreign body was recognized. The audible slap and palpable thud characteristic of foreign bodies in the trachea, as described by Chevalier Jackson, were noted in four of the patients (both of the beads, the pebble, and the grain of corn).

In all, 32 (12.8 percent of the series) of the foreign bodies were removed with guidance of the biplane fluoroscope. These included 16 of the 17 open safety pins in the esophagus, as well as the 4 foreign bodies in the stomach (the Alnico magnet was used in removal of two of the bobette pins). The biplane fluoroscope was also used in all of the patients with pointed metallic foreign bodies in the bronchus (open safety pin, baby pin, common pin, screws, nail, tack, dental root canal reamer) and in 4 of the other cases of foreign bodies in the bronchus (tooth cap, steel shaving, lead shot, tooth).

There were two fatalities in this series, so that the mortality for the entire group of 250 patients was 0.8 percent. One of the fatalities, the only case in which tracheotomy was required following bronchoscopy for foreign body, was in a child of 13 months who had inspired a piece of peanut shell and was first seen about seven hours later with considerable dyspnea, moderate inspiratory stridor and expiratory wheeze. Six hours following bronchoscopic removal of the foreign body (4 mm. bronchoscope; 2 minutes 9 seconds) laryngeal dyspnea had increased considerably and tracheotomy was performed after preliminary introduction of the 4 mm. bronchoscope with only partial relief of the dyspnea. While the child was being fluoroscoped about 12 hours later, a complete left pneumothorax, secondary to mediastinal emphysema, developed rapidly, and death occurred before equipment for aspiration of the pleural space could be obtained. It is possible that the pneumothorax in itself might not have been fatal, but the autopsy showed that there were, in addition, multiple areas of lobular atelectasis throughout the right lung.

The other fatality was in a negro male of 47 who had complained of substernal pain after eating spare-ribs about 24 hours before he was first seen. The general physical examination on admission was essentially negative except for some elevation of temperature (99.6° F.); the leukocyte count was 15,000, with 89 percent polymorphonuclears, of which 23 percent were nonfilaments. Although roentgen examination of the esophagus was negative, the symptoms appeared to warrant esophagoscopy. A sharp, double-pointed spicule of bone, 3.5 cm. in length, was found lying transversely at the level of the aortic arch with one extremity imbedded in the left wall of the esophagus; the bone was removed. The temperature subsided within three days; roentgen examination five days after removal was negative, and the leukocyte count was 7,000 with 70 percent polymorphonuclears. The patient was discharged, asymptomatic, on the sixth day following removal of the foreign body. Four days later he returned, having had a large hematemesis; although the bleeding apparently ceased for a few hours following his readmission, a second massive hemorrhage was fatal. The autopsy

showed a small fistulous communication between an eroded area 1 mm. in diameter in the wall of the upper portion of the descending aorta and the esophageal lumen. The presence of spontaneous perforation prior to esophagoscopy is suggested by the elevated temperature and leukocyte count on admission. The location of the foreign body was such that one of the sharply-pointed extremities was directed toward the pulsating aorta, and the pathologist suggested the possibility of pressure necrosis of a small area of the aortic wall. Clerf reports three cases of esophageal foreign body in which death was attributed to pressure necrosis of the wall of an adjacent large vessel. In such cases as in the one just described, the fatal hemorrhage occurred some time after removal of the foreign bodies (11 days after removal of a jackstone, 12 days after removal of an open safety pin, and 36 hours following removal of a fish bone).

In none of the patients with foreign body in the hypopharynx, esophagus or stomach, except for the one mentioned above, was there permanent damage resulting from the sojourn of the foreign body or the endoscopic procedure by which it was removed. In the several cases in which a coincident esophageal lesion was responsible for lodgment of the foreign body, the prognosis does not appear to have been affected in one way or the other by the temporary presence of the foreign body.

There were, however, several patients with foreign bodies in the bronchus in whom prolonged sojourn of a foreign body had resulted in irreversible changes. In one of these patients a baby pin had been present in the right lower lobe bronchial tree for 21 years; productive cough and blood streaking persisted despite removal of the foreign body, and the bronchogram showed extensive saccular bronchiectasis. Lobectomy appeared indicated and was performed with uneventful convalescence. Lobectomy also was done in one of the two patients with broncholithiasis in whom prolonged obstruction had resulted in atelectasis and advanced bronchiectasis; although the bronchogram following removal of the broncholith in the other patient showed some bronchiectasis, the symptoms did not appear sufficient to require resection.

There were three other patients in whom persisting abnormal density was evident on x-ray examination following removal of a foreign body from the bronchus (tooth cap in lower lobe bronchus for eight months, chicken bone in lower lobe bronchus for six weeks, and chicken bone in lower lobe bronchus for period undetermined), but these patients are essentially asymptomatic. (Ann. Otol., Rhin. & Laryng., Dec. '48, C.M. Norris)

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Carcinoma Cells in Sputum and Bronchial Secretions: The occurrence of grossly visible fragments of neoplastic tissue in the sputum of patients with cancer of the lung has been observed for many years. While such an occurrence

is rare and usually indicates far advanced disease, an occasional diagnosis of bronchogenic carcinoma has been made by embedding and sectioning the tumor tissue so obtained. Betschart in 1895 reported a case of bronchogenic carcinoma in which the diagnosis was made by section of tumor fragments present in the sputum and cited 3 previous reports of cases in the literature. Similar diagnoses based on gross particles or sputum section were subsequently reported by a number of investigators. In general the results were not considered of great value in the diagnosis of bronchogenic carcinoma. The first report of the cytologic examination of sputum in a case of bronchogenic carcinoma was made by Hampeln in 1887. He found large atypical epithelial cells in the sputum which he believed were diagnostic of carcinoma.

Papanicolaou for many years has stressed the value of the smear test as a basis for the diagnosis of malignant neoplasms having a free surface. Although primarily concerned with cytologic study of vaginal secretions in the diagnosis of uterine cancer, he has reported results of sputum examination in 24 patients with carcinoma of the lung. In 22 (88 percent) of these patients, the sputum smears were positive for malignant cells. No false positive reports were given in this series.

After a preliminary orientation study, specimens of sputum or bronchial secretions were studied as unknowns and positive or negative results were reported. In doubtful cases, a second or even a third specimen was examined. In all, sputum or bronchial secretions were examined for carcinoma cells from a total of 1,600 patients. In 150 of these patients results were reported as positive and in 1,450 they were considered negative. No follow-up studies were done on the group of patients for whom the results were negative but the vast majority were considered to have nonneoplastic lesions. After positive results in 150 consecutive cases were reported the results were tabulated and analyzed.

The technic employed was based on the original work of Dudgeon and Wrigley (1935). Sputum was examined while fresh or it was collected in a few cubic centimeters of 95 percent alcohol. Five smears were made from each specimen. The smears were fixed, while wet, in equal parts of absolute alcohol and ether for 30 minutes. The staining method used routinely is Harris' hematoxylin with dilute eosin as a counterstain. It has proved entirely satisfactory for nuclear detail, the factor on which the diagnosis is primarily based, and has the advantage of familiarity to pathologists. Five smears from each specimen are fixed and stained. The films are then carefully scanned under low power, the high dry objective being used to study cell detail. It may be useful to mark suspicious cells or clumps with ink and later to return for careful consideration of these areas. The number of carcinoma cells per slide in a case of carcinoma varies considerably. In rare cases almost every low power field will show cancer cells. In many cases each slide will contain a few scattered clumps and isolated single

cancer cells. Occasionally only one or two of the five slides will give positive results. For this reason it is important that all slides in a given case be examined methodically, a mechanical stage being used if necessary. The time required for examination of films varies inversely with the number of atypical cells present. In some cases a positive diagnosis may be made almost at a glance or after 2 or 3 minutes' search; in others, in which few cells are present, 20 or 30 minutes may be required. From 20 to 30 minutes should be spent in a case in which the results are negative.

Smears of sputum or bronchial secretions from nonneoplastic diseases of the lung revealed squamous and ciliated columnar epithelial cells, macrophages, and various inflammatory cells. Carcinoma cells may be distinguished from normal cells in the smear by a variety of atypical features including large size, variation in size and shape particularly of the nuclei, hyperchromatism, and large nucleoli. A high degree of accuracy of diagnosis may be accomplished by this method as is shown by the occurrence of only 3 proved false positive reports in 150 cases in which a diagnosis of carcinoma was made by the smear technic.

The appearance of carcinoma cells in sputum and bronchial secretions varies with the histologic type of tumor in the bronchus. Cancer cells originating in a bronchogenic carcinoma of small cell oat-cell type or in a keratinizing squamous cell type provide the most distinctive morphologic characteristics. A diagnosis of the histologic type of tumor present was usually possible in the case of these two cell types. In most other bronchogenic carcinomas, especially the more undifferentiated tumors, a diagnosis of carcinoma cells present was made without reference to histologic type. Metastatic lesions of the lungs may be the source of carcinoma cells in sputum or bronchial secretions.

The interpretation of smears requires considerable experience but, once this is gained, the test has great practical value. In practice a microscopic diagnosis of carcinoma is frequently established within an hour of receiving the specimen at the laboratory. It provides microscopic evidence of cancer in many cases in which proof is otherwise not obtainable.

Many bronchogenic carcinomas, particularly lesions of the upper lobe and peripherally located neoplasms, are not accessible for bronchoscopic biopsy. In this series 114 patients underwent bronchoscopic examination. In 17 of these, material for biopsy could not be obtained. In 31 cases the tissue removed for biopsy proved to be nonneoplastic while in the remaining 66 cases a microscopic diagnosis of bronchogenic carcinoma was made on the tissue removed. Any method which serves to supplement bronchoscopic examination and provide microscopic proof of the presence of carcinoma when biopsy cannot be performed is of immense practical importance in the diagnosis of pulmonary neoplasms.

It has been shown that cytologic study of sputum and bronchoscopically removed secretions provides this microscopic proof in the majority of these inaccessible lesions. By combining both methods in the examination of the patient with suspected bronchial carcinoma a much higher rate of positive diagnoses can be accomplished than heretofore has been the case.

The ability to make a microscopically proved diagnosis of cancer in a higher percentage of cases will serve to reduce materially the preoperative diagnosis of indeterminate pulmonary lesion. In this series, exploration for bronchogenic carcinoma was carried out in a total of 58 cases. In 31, or 53.4 percent, carcinoma was diagnosed on the basis of bronchoscopic biopsy. In 25 cases, cancer cells in the sputum or secretions provided the only microscopic evidence of malignancy. In 2 cases a false positive diagnosis was given on the basis of smears.

Furthermore, by use of the smear technic, a positive microscopic diagnosis of carcinoma may be made in many cases of suspected bronchogenic carcinoma in which a bronchoscopic examination is not indicated. In 32 cases no bronchoscopic examination was carried out. The demonstration of carcinoma cells in the sputum in these cases was a valuable confirmation of the clinical and roentgenologic evidence of primary bronchogenic carcinoma.

The need for earlier diagnosis of cancer of the lung is shown by the low rate of resectability of these lesions. In this series 24 bronchogenic carcinomas were removed in a total of 143 cases of suspected malignant disease. More widespread use of cytologic examination of sputum for cancer cells offers a hope of earlier detection of bronchogenic carcinoma before the lesion has progressed to an inoperable stage. Because of the vital importance of earlier diagnosis, it is felt that every patient with an undiagnosed lesion of the chest should be given the benefit of a carefully conducted cytologic examination of sputum or bronchial secretions for cancer cells. (Surg., Gynec. and Obstet., March '49, L. B. Woolner and J. R. McDonald)

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Tissue-Culture Evaluation of the Viability of Blood Vessels Stored by

Refrigeration: Although a graft of dead bone, cartilage, or blood vessel, may provide a suitable framework and produce a functional result the evidence at hand indicates that in the case of arteries, at least, more reliable results are obtained when living tissue is employed. Consequently, efforts have been directed primarily to finding a method of preserving vessels in a viable state. Several modes of storage, including freezing, have been abandoned by the authors at least temporarily, because when employed, viability could be demonstrated only sporadically and, in experimental animals results from grafts were poor. The method of storage recommended by the authors is not entirely new and is based on the work of many others.

Bert refrigerated rats' tails in confined air at about $+12^{\circ}$ C. for several days and then successfully transplanted them. Carrel stored blood vessels, skin, fascia, and other tissues, in vaseline, moist air, normal saline, and Ringer's solution, at temperatures slightly above freezing. He evaluated his method of preservation after from one to 6 days by cultivation of stored embryonic tissue and after intervals as long as 7 weeks by grafting stored adult tissue in animals. The maximum duration of cell viability was not determined. Other workers studied the survival of refrigerated embryonic and other tissues by tissue cultivation or oxygen consumption, but were either unable to demonstrate viability after more than short intervals or were concerned with comparatively brief periods of storage. Their work indicates that refrigerated tissue retains its viability for varying periods of time, depending primarily on the type of tissue, and secondarily on the size of tissue pieces, available oxygen, storage temperature, and other factors. Irreversible changes took place quickly in brain, kidney, liver, and other solid organs, whereas bone, cartilage, skin, fascia, and blood vessels, survived for comparatively long intervals. The degree of preservation was more or less dependent on the size of the fragments, very small pieces of tissue surviving for only brief periods. Oxygen demand was greatly diminished as the temperature fell. Tissue fragments at 37° C. required very high oxygen tensions to prevent necrosis; at 0° C. tissues were able to survive for a time even when oxygen was excluded.

Tissues for clinical grafting have been stored refrigerated for short intervals. La Roe kept skin in Tyrode's solution for periods of one week; Castroviejo stored corneas for from two to 5 days in Ringer's or normal saline. Both reported satisfactory clinical results. More recently, Matthews preserved human skin wrapped in saline sponges, in air-tight containers at from 3° to 6° C. He reported viability after eight days as measured by tissue cultivation, and stated that autogenous grafts at three months were successful.

It is thus clear that certain tissues can be maintained in a vital state by simple refrigeration. However, evidence concerning the limitations of storage methods is slowly accumulating. Skin and blood vessels can be preserved alive for about a week in the absence of a buffered environment by storing at 0° C. with the exclusion of oxygen. As shown both by other workers and the authors, however, there is an unfavorable accumulation of acid metabolites and a rapidly falling viability. Tissue stored in such media as BSS (balanced salt solution), Ringer's solution, or normal saline, at temperatures just above 0° C., will also remain alive for short periods, as demonstrated by the authors' data, but the lack of suitable nutrient material is evidently unfavorable for lengthy preservation of viability. In addition, blood vessels so kept may become edematous and prove unsuitable for vascular grafts after as little as 24 hours' storage. Preservation in serum or whole blood is also satisfactory for short periods of time, but the large protein and fat molecules seem seriously to interfere with metabolism. The preservation of tissue in 100 percent serum was unsuccessful in one series of experiments performed by the authors.

It appears that an ideal storage medium should contain a physiological concentration of salts, buffer, glucose, and the small accessory molecules which are present in serum. The large protein and fat molecules should probably be in reduced concentration. The storage medium developed by Hanks and Wallace fulfills these criteria. It was found by them to be entirely suitable for storage of rabbit skin for periods up to two weeks at 8° C. Their procedure was modified by the authors by employing somewhat lower temperatures and by the more liberal use of storage medium.

The use of serum ultrafiltrate as a storage medium is suggested because all of the large molecules have been removed from it, and the small molecules remain. The data accumulated to date indicate that this solution either alone or with 10 percent serum is satisfactory but is not superior to 10 percent serum in BSS.

In the early part of the work cotton stoppers were used in the storage flasks because it was believed to be important to facilitate the metabolic exchange of the blood vessels which was evident even at low temperatures. This proved fairly satisfactory except in the case of small vessel segments which retained viability for only relatively short periods of time. Experiments conducted with tightly-stoppered flasks showed that the metabolic activity was not sufficient to require a free exchange of carbon dioxide and oxygen. In addition, it was found that small vessels preserved in this latter manner remained viable as long as did large ones. Consequently, tightly-fitting stoppers are now used routinely.

The results of the present study in which the authors employed tissue-culture procedures indicate that blood vessels may readily be preserved alive and with normal physical properties for periods of at least seven weeks through the use of 10 percent serum in BSS with 5 percent of a solution containing 1000 micrograms of streptomycin and 1000 units of penicillin per ml. and refrigerated at temperatures just above freezing. The preserved segments of arteries so stored closely resemble fresh vessels in physical qualities, and a large series of successful grafts in dogs has been completed. Vessels have also been collected from human beings, stored, and have been employed successfully for alleviation of the tetralogy of Fallot and in the treatment of coarctation of the aorta. The authors state that this method of blood-vessel preservation appears superior to any other yet tried by them including freezing.

The general method of storage with suitable modifications might be applicable to fascia, skin, bone, nerves, cartilage, and corneal tissue.

The BSS used in this study was supplied by Dr. J. H. Hanks. It resembles Tyrode's solution and was prepared as follows:

Stock solution, contents per 250 cc.: NaCl 20 Gm., KCl 1 Gm., $MgSO_4 \cdot 7H_2O$ 0.2 Gm., $MgCl_2 \cdot 6H_2O$ 0.2 Gm., $CaCl_2$ 0.35 Gm. (dissolved separately), Na_2HPO_4 0.15 Gm. (0.38 Gm. of $Na_2HPO_4 \cdot 12H_2O$), $KHPO_4$ 0.15 Gm., glucose 2.5 Gm., 0.4 percent phenol red 12.5 cc.

Buffer: 1.4 percent $NaHCO_3$.

The stock solution was stored at room temperature with 1 cc. chloroform. The final solution was made by diluting the stock 1:10, autoclaving, and adding 0.5 cc. (previously autoclaved) buffer per 20 cc. This was stored in cotton-stoppered containers in the icebox, which caused pH equilibration at about pH 7.6. A somewhat similar solution prepared according to Simms' formula is available commercially from Microbiological Associates, Flemington, New Jersey. (Ann. Surg., March '49, E.C. Peirce et al.)

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Effect of Hesperidin Methyl Chalcone (Vitamin P) on Diabetic Retinopathy:

Under proper dietary management and with the aid of insulin therapy, most diabetic patients can be kept in a reasonably normal state of nutrition and in satisfactory metabolic balance as far as gross glycosuria and freedom from frank symptoms of diabetes are concerned. Nevertheless, these clinical criteria of control of the disease have proved inadequate to protect the diabetic patient against the onset of vascular disease if he lives long enough. This fact has recently been emphasized by Dolger who showed that in a group of 200 patients whose diabetes was of 25 years' duration, there was evidence of vascular disease in every case. Dolger concluded that in diabetes the accelerated vascular damage is an associated phenomenon and not a complication. That this finding may not be invariable is indicated by 2 patients in the Indianapolis series, whose juvenile diabetes has extended well over 20 years, and who show no demonstrable retinal, renal, or other vascular damage whatsoever. How to prevent or retard vascular degeneration is a problem and a challenge.

The most common and in many cases the earliest manifestation of vascular involvement is the appearance of hemorrhages and exudates in the retina. There have been no satisfactory methods of treatment of these retinal lesions. If the progressive course of diabetic retinopathy could be arrested, similar damage to the vascular system in other parts of the body might likewise be favorably influenced.

The early investigations by Szent-Gyorgyi on vitamin C led him to believe that a substance other than ascorbic acid was responsible for the petechial hemorrhages which appear in deficiency states. He identified the substance

as a mixture of the flavone glucosides of hesperidin and eriodictyol. There was evidence that the absence of this substance increased capillary permeability and so it was called vitamin P. The present study was undertaken to determine the effect of vitamin P on the retinal hemorrhages of diabetes. The vitamin P used was hesperidin methyl chalcone (H.M.C.); it is derived from the rinds of citrus fruits. (Rutin, another of the flavone glucosides, also has vitamin P activity, differing from hesperidin methyl chalcone in that its potency, according to Scarborough, is about one-half that of pure hesperidin.) As a part of the investigation additional information was sought in the hope of throwing some light on other factors which might be associated with the appearance of retinal hemorrhages. It is well known that diabetic retinopathy tends to be progressive. There is a wide variation in the rate at which fresh hemorrhages and exudates appear, and these lesions may undergo spontaneous absorption for no apparent reason. It is difficult, therefore, to evaluate fully any therapeutic agent unless a large series of cases is followed for a long period. The number of patients so followed in the authors series is too small to be of statistical significance, but some information has been obtained by a careful study of individual cases.

Twenty-two diabetic patients with retinal hemorrhages have been treated with hesperidin methyl chalcone. The daily dose was small in the beginning but was gradually increased when it became apparent from studies of the effects of administration of massive doses by Kirtley and Peck that there were no side reactions from the drug. All but one of the patients ultimately received between 100 and 300 mg. of the drug daily, and in some instances these doses were exceeded considerably for short intervals.

Systematic examinations of the fundus oculi were made through a dilated pupil by the same examiner (M.M.) at periods of from 2 to 3 months. Each patient was followed for an average time of 15 and 1/2 months, the shortest period being 4 months, and the longest 32 months. Every patient showed one or more of the typical types of retinal hemorrhages seen in diabetes. Particular attention was given to the hemorrhagic lesions because capillary damage may be responsible for their appearance. If the exudates seen in diabetic retinopathy represent the extravasation of plasma from vessels, they may also be a manifestation of increased capillary permeability. On the basis of ophthalmoscopic findings the retinal lesions were arbitrarily divided into 2 groups, early and moderate. Those patients with punctate hemorrhages which did not exceed 5 in number, with or without small waxy exudates, were classified as having early diabetic retinopathy. In patients in whom punctate hemorrhages were more numerous and larger deep hemorrhages were present, the retinopathy was classified as moderate. Many of the patients in the latter group showed extensive areas of retinal exudation and a few had cotton wool patches.

As part of the study prothrombin time and capillary fragility were measured. The level of plasma proteins was also determined. Although the number of

patients observed over a sufficiently long period was not large (23 in all), the following conclusions seem justified.

There was no significant alteration in the plasma albumin or globulin of 23 diabetics. All but 3 had some evidence of diabetic changes in the retina. The prothrombin time tended to increase in those patients whose known duration of the disease averaged 10 years. As would be expected these patients also showed more extensive changes in the retina. The relationship, if any, between prothrombin time and diabetic retinopathy deserves further study. In about 50 percent of the cases of retinopathy in this group increased capillary permeability, as manifested by the petechial index, was displayed. There did not appear to be any correlation between capillary permeability and prothrombin time. There was improvement in the petechial index in a certain number of patients after the administration of vitamin P, suggesting that this vitamin has some effect on capillaries. In most patients in whom this was observed there was no significant alteration in the appearance of retinal hemorrhages. Retinal hemorrhages are seen in diabetics who do not show increased capillary fragility. Vitamin P did not alter the course of diabetic retinopathy in a majority of these patients. There were 6 whose lesions progressed, 5 who showed improvement, and 5 others have benefited. Any favorable effect attributed to vitamin P must take into consideration the possibility of spontaneous changes in retinal lesions of diabetes. (Am J. M. Sc., March '49, F. B. Peck and M. Mann)

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Aminopterin (A Folic Acid Antagonist) in the Treatment of Leukemia:

In 1948, Meyer reported temporary clinical and hematological improvement in some patients with acute leukemia treated with the folic acid antagonists, pteroyl aspartic acid (An-Fol-R) and methyl pteric acid (Met-Fol-B). Further studies with these 2 compounds and a third antagonist, 4-amino-pteroyl-glutamic acid (aminopterin) have been presented. More recently, Farber et al. (Medical News Letter of 2 July 1948) have reported their experiences with aminopterin in 16 cases of acute leukemia in children. These investigators reported clinical and hematological improvement in 10 of their patients.

A collaborative study was undertaken by the authors and several investigators in other institutions. Forty-three patients with leukemia were treated with aminopterin. The types of leukemias were classified as follows: 21 myeloid (3 chronic, and 14 acute in adults, and 4 acute in children) and 22 lymphoid (3 chronic and 7 acute in adults, and 12 acute in children). Of these 43 patients, 4 (9.3 percent) showed improvement in hematological and clinical pictures. The remainder showed no change or were adversely affected. The 4 improved patients were children, 3 with the acute lymphoid type and 1 with the acute myeloid type. No beneficial results were seen in adults. Patients with lymphoid leukemia, to whom aminopterin was administered, responded more rapidly and precipitously with peripheral leukopenia and bone marrow hypoplasia than those in the myeloid group.

Aminopterlin had no beneficial effect on the anemia present in 41 patients, but 2 did show improvement in hemoglobin and red blood cell levels during treatment. The effect of the drug on the white blood cells varied. In 18 patients the leukocyte count was increased or unchanged, in 17 moderate leukopenia developed and in 4, severe leukopenia. In all these patients differential values were not changed with treatment. Aminopterlin appeared to have no effect on the blood platelet count.

Depression of the bone marrow was severe at times. In 2 patients there was moderate reduction in cellularity, and in 9, severe irreversible hypoplasia occurred. There was no detectable change in the bone marrow of 29 subjects. Of 28 febrile patients, 4 showed reduction in temperature following treatment. Enlarged lymph nodes were found in 25 patients. Of these, there was reduction in size in 6, no change in 15, and progressive enlargement in 4. Hepatomegaly was encountered in 23 persons; 3 showed reduction in size, 1 an increase, and 19 no change. Splenomegaly was found in 28 subjects, 6 showed reduction in size of the spleen, 2 an increase and 20 no change. Loss of hair occurred in 3 patients. Hemorrhage into the skin, associated with severe leukopenia and hypoplastic bone marrow, was noted in 2 others. Stomatitis was observed in 12 patients, hematemesis in 6, and rectal bleeding in 7.

The toxicity of aminopterlin deserves emphasis. Leukopenia with severe irreversible hypoplasia of the marrow was the most serious complication. This toxic effect was more common in those patients receiving the drug for longer periods of time. Total dosage and dosage per body weight could not be related to marrow hypoplasia. Daily blood counts must be made and patients followed carefully because a patient with a leukocytosis of from 70,000 to 100,000 cells per cu. mm. may suddenly become severely leukopenic within a period of from 24 to 72 hours. Mucous membrane lesions were the most frequent and most troublesome of the toxic signs. Shallow ulceration of buccal and pharyngeal mucosa with the formation of an adherent dirty gray overlying membrane on an erythematous base was commonly seen. Hematemesis and rectal bleeding were also noted and were believed to be expressions of similar mucosal lesions within the gastrointestinal tract. The mucous membrane lesions occurred haphazardly and could not be related to dosage or duration of medication. Liver extract was not beneficial. In a few patients with lesions of a mild nature appearing early in the course of therapy, aminopterlin was continued and no further progression of these lesions was noted. When the lesions were of serious nature withdrawal of the drug led rapidly to mucous membrane healing. Alopecia was occasionally seen. This complication was not considered cause for cessation of therapy. A maculo-papular hemorrhagic skin rash was sometimes encountered. The rash usually accompanied severe aminopterlin toxicity with leukopenia, marrow hypoplasia and mucosal lesions.

The most striking findings in this study were the unpredictability of the action of aminopterin and the very evident toxicity of the drug. Both beneficial and toxic effects could not be controlled. Nor could these effects be related to dosage of aminopterin alone or to its administration simultaneously with liver extract, terofterin, or other supportive measure. (Am. J. Clin. Path., Feb. '49, L. M. Meyer et al.)

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Use of a Folic Acid Antagonist, Aminopterin, in Chronic Leukemia: This study is based on observations of the effect of the folic acid antagonist, 4-amino-pteroyl-glutamic acid (aminopterin) in nine patients with chronic leukemia. One of the patients having myelogenous leukemia received two series of treatments. The drug was taken orally, in daily doses ranging from 1.25 to 7.5 mg. over periods of from 6 to 25 days.

Farber and co-workers used 4-amino-pteroyl-glutamic acid in 16 children with acute leukemia. In 10 they observed temporary clinical and hematological improvement. Similar but less striking results have been reported by others. Meyer observed no hematological response following the use of folic acid antagonists in 3 patients with chronic lymphatic leukemia. Judging from the comparison which can be made from observations on acute leukemia, in which numerous stem cells are present in the blood, and Meyer's patients with chronic lymphatic leukemia, in which condition few, if any, stem cells, are found, it seems evident that stem cells and other immature leukocytes are more sensitive to the folic acid antagonists than are the more differentiated cells.

In this study the authors observed that stem cells in both myelogenous and lymphatic leukemia, and immature granulocytes in myelogenous leukemia decreased in number, but platelet and erythrocyte counts varied. Usually, mature granulocytes decreased in number during treatment, but lymphocytes of mature type were relatively unaffected. These observations indicate that, in addition to the morphological similarity of lymphoblasts and myeloblasts, there are also functional similarities in the response to folic acid antagonists. Progressive falls in total leukocyte counts occurred for as long as 10 days after treatment was stopped. The most important morphological effects included the appearance of small numbers of megaloblasts in material aspirated from the sternal marrow. The cells were indistinguishable from those characterizing erythropoiesis in Addisonian pernicious anemia in relapse. Usually they were seen after approximately from 50 to 100 mg. of the medication had been ingested over periods of from 7 to 23 days.

In 2 patients with lymphatic leukemia there was a decrease in the sizes of lymph nodes. The hematological effects of therapy were temporary and never completely adequate because treatment had to be stopped when toxic signs appeared.

The toxic effects, in the order of appearance, included pharyngitis, stomatitis, sore tongue, crampy abdominal pain sometimes associated with diarrhea, dermatitis, and alopecia. Usually pharyngitis first appeared within a few days before or after significant changes in the leukocyte counts were noted. The total dosage of the drug which produced toxic effects varied from 30 to 75 mg., although one patient received 105 mg. without experiencing such signs. There was evidence that when small doses are used initially, a larger cumulative dose can be tolerated before toxic symptoms appear. Autopsies of two patients, who expired shortly after therapy was stopped, revealed no changes which could be attributed to the medication.

Although 4-amino-pteroyl-glutamic acid has a definite hematological effect, there was no subjective improvement in any of the patients. The use of the drug in chronic leukemia is limited by the early development of toxic phenomena. (Am. J. Clin. Path., Feb. '49, L. Berman et al.)

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Studies of the Human Colon - I. Variations in Concentration of Lysozyme with Life Situation and Emotional State: Lysozyme, a bacteriolytic and mucolytic enzyme, was first described by Fleming in 1922, when he was studying the anti-bacterial properties of tears. Since then the enzyme has been shown to be present not only in human tears but also in nasal mucus, gastric juice, the secretions of the large and small intestines, and human milk. All pathological transudates and exudates contain large amounts. The richest source of this lytic enzyme is the white of hen's eggs. Further studies on this substance have shown it to be a basic protein or polypeptide containing about 16 percent nitrogen and 2 or 3 percent sulphur. It is of small molecular size, having a diameter of less than 30 millimicrons. It is soluble and heat stable in acid solution, but insoluble and heat labile in alkaline solutions.

Recent studies on the substrate of this enzyme by Karl Meyer and his associates have shown that the enzyme depolymerizes certain high molecular weight amino carbohydrates which they have isolated from the susceptible organisms (Micrococcus lysodeikticus or Sarcina lutea). However, the search for the substrate of lysozyme in various organs of the body has thus far been fruitless. Lysozyme concentrations can be measured by determining the rate of clearing of opaque suspensions of the susceptible organisms or by determining the rate of depolymerization of the substrate isolated from the susceptible organism by the viscosimetric method of Meyer.

Meyer has further shown that the lysozyme concentrations are very high in the gastric juice of patients with peptic ulcer and in the stools of patients with chronic ulcerative colitis. He has demonstrated that lysozyme will digest mucus and postulates that the destruction of the protective coating of the bowel exposes

the underlying mucosa to the action of noxious agents of the intestine such as hydrochloric acid or the indigenous bacterial flora. These postulates he has substantiated in part by feeding lysozyme to dogs and thus producing acute ulceration of the upper gastro-intestinal tract.

Because of these findings, it seemed profitable to explore variations in lysozyme concentration in the stools of human subjects under a variety of circumstances and life situations. All tests were done according to the viscosimetric method of Meyer. The range of lysozyme concentration observed among all the groups of subjects are summarized in the table below.

	Units per gm. Wet Stool.
Normal Subjects	0.3-- 1.7
Acute Congestive Heart Failure	1.2
Cancer of the Large Intestine	3.2
Mucus Colitis, (Mild Cases)	
A) Constipation	0.6
B) Diarrhea	0.4-- 1.5
Ulcerative Colitis	
Remission	0.7-- 1.6
Mild Symptoms	13. -- 25.
Mod. Severe Symptoms	40. -- 100.
Regional Enteritis, Remission	0.4-- 0.8
Acute (24 hour) Gastroenteritis	0.7

It would appear that in normal subjects there may occur a rise in colonic lysozyme concentrations in response to situational threats productive of anxiety and apprehension and during periods of anger, hostility, and resentment. The increase in lysozyme concentration in these subjects was not of great magnitude, however; neither was it sustained over a period of days. Being thus of minor degree and transitory, it was probably of little importance to the welfare of the individual.

In a series of 12 patients with ulcerative colitis in a state of remission, with minimal symptoms and enjoying feelings of relative relaxation and security, the stool lysozyme concentrations were low (0.7 to 1.6 units per Gm.). In 4 patients with ulcerative colitis whose symptoms were mild, stool concentrations varied from 13 to 25 units per Gm. In 3 subjects with ulcerative colitis whose symptoms were moderately severe, concentrations of stool lysozyme varied from 40 to 100 units.

In subjects with ulcerative colitis in whom day to day observations were made, lysozyme concentration was low during remissions which coincided with periods of relative self-assurance and security. During exacerbations, however, usually

marked by situations provocative of unexpressed anger, hostility, and resentment, there occurred sharp rises in stool lysozyme. In fact a sustained, marked elevation of lysozyme concentration usually presaged a period of bloody diarrhea.

In 6 patients with mild mucous colitis, both those with diarrhea and those with constipation, lysozyme concentration was low (0.4 to 1.5). In one subject with moderately severe constipation and mucous colitis, lysozyme concentration was in the neighborhood of 25 during times of anger, hostility, resentment. During periods of relative security, lysozyme in the stool fell to lower levels. One subject with mucous colitis displayed fairly marked changes in lysozyme concentration which corresponded to changes in symptomatology and life situation.

The possibility that ulceration itself might provoke an elaboration of lysozyme was further tested by analyzing the stools of a subject with an ulcerated carcinoma of the colon. Lysozyme concentration was, however, persistently low (3.2).

Attempts to assess the effects of lysozyme applied to the intact colonic mucosa were made in a subject who was healthy except for a colostomy through which had herniated a large segment of sigmoid colon. Human tears containing lysozyme in a concentration of 600 units per cc. were applied to the surface of the bowel on small cotton pledgets and allowed to remain in place for 24 hours. At the end of this time, in 3 of 4 such experiments, a small, sharply circumscribed area of inflammation and edema was noted at the point of application. Control applications of normal saline, boiled tears and dry cotton failed each time to produce any lesion. The fact that unboiled tears are capable of bringing about injury to the mucous membrane of the normal human colon, while boiled tears and saline fail to do so, suggests that the lysozyme may be responsible for damaging the colonic mucosa, or, by removing the protective mucous coating, for exposing the mucosa to injury from trauma or infection. This finding in human beings is in keeping with the data of Meyer in which intestinal ulcers were produced in animals by feeding lysozyme. An analogous situation had been produced in cats by Rappaport and Nauss, who found that the colon, damaged by chemical agents was much more susceptible to the invasion of Entamoeba histolytica. It is possible that in human beings the mucolytic action of lysozyme allows amebae as well as other noxious agents freer access to the cells lining the wall of the bowel.

That lysozyme is not the result of ulceration in the bowel is established by finding it in high concentration in the stools of patients who have no ulcerations in their intestine, as well as by finding variations in lysozyme concentration in the secretions removed from the surface of the intact mucosa. Likewise, low values were found in the stools of patients with ulcerating neoplastic disease of the large intestine. Moreover, in the subjects with ulcerative colitis described, the rise in colonic lysozyme antedated the onset of bleeding and other symptoms.

It is concluded from these data that lysozyme concentration in bowel secretions is related to the occurrence of ulceration, and that furthermore, variations in the production of lysozyme correspond to variations in the state of security of the organism as a whole. When the individual was meeting threats to his security arising out of problems of day to day adjustment with feelings of humiliation, guilt, anger and hostility, but was repressing them to present a serene and nonaggressive exterior, an excess of colonic lysozyme was likely to be elaborated. (Am. J. M. Sc., March '49, W. J. Grace et al.)

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List of Recent Reports Issued by Naval Medical Research Activities:

Naval Medical Research Institute, NNMC, Bethesda, Maryland

<u>Project</u>	<u>Report No.</u>	<u>Date</u>	<u>Title</u>
NM 001 008	3	21 July '48	An Apparatus for the Rapid Determination of the Relative Luminosities of Fluorescent Solids Particularly Applicable for Field Work
NM 005 007	Addendum to 4	30 Dec. '48	An Entomological Survey of Ponape, Eastern Caroline Islands
NM 005 004 X-535	20	18 Nov. '48	The Susceptibility of Albino Rats to <u>Schistosoma Mansoni</u>
NM 005 004 X-535	21	8 Feb. '49	The Susceptibility of Golden Hamsters to <u>Schistosoma Mansoni</u>
NM 005 007	6	5 Oct. '48	The Cyclic Transmission of Japanese Encephalitis by <u>Culex Quinquefasciatus</u> , Utilizing Young Albino Mice
NM 005 007	7	14 Oct. '48	An Epidemic of Eosinophilic Meningitis, A Previously Undescribed Disease, Occurring on Ponape, Eastern Carolines
NM 005 019	1	10 Dec. '48	The Effects of Larval Population Density on Some Laboratory Characteristics of <u>Anopheles Quadrimaculatus</u> , Say

NM 007 022	1	30 Aug. '48	A Study of the Histologic Structures of the Cervix Immediately Post-Partum
NM 007 024	5	8 Mar. '49	Failure of Adrenocortical Extract to Modify the Immunity Acquired by Intact Mice Through the Use of Pneumococcal Vaccine
NM 011 013	3	13 Jan. '49	Studies of the Acute Toxicity of Gallium

Naval Medical Research Institute, NNMC, Bethesda, Maryland (Cont.)

<u>Project</u>	<u>Report No.</u>	<u>Date</u>	<u>Title</u>
NM 011 013	4	25 Jan. '49	Studies of the Metabolism of Gallium
NM 013 012 X-182	3	23 Feb. '49	A Modified Procedure for the Preparation of the Lucite Calvarium in Monkeys

Medical Research Laboratory, U.S. Naval Submarine Base, New London, Conn.

NM 003 008 X-423	1	Dec. '48	Checkerboard Visual Acuity Targets: An Experimental Validation
NM 003 011 X-493	4	15 Aug. '48	A Factor Analysis of Acuity and Phoria Measurements Obtained with Commercial Screening Devices and by Standard Clinical Methods
NM 003 011 X-493	5	30 Aug. '48	Comparative Performance of Commercial Screening Devices and Far and Near Wall Charts Utilizing the Same Test Targets
Color Vision Report	4	22 Nov. '43 Reissued 1 Dec. '48	The Effect of Certain Illuminants on Scores Made on Pseudo-Isochromatic Tests

Medical Field Research Laboratory, Camp Lejeune, N.C.

NM 005 031	-	21 Jan. '49	Biological and Crossbreeding Studies on <u>Aedes Hebrideus</u> and <u>Aedes Pernotatus</u> (Diptera: Culicidae)
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NM 012 008 - 9 Feb. '49 Evaluation of Urinary Dextrose and Albumin Data as Derived from the Use of Specific Commercial Solidified Reagents

U.S. Naval School of Aviation Medicine and Research, NAS, Pensacola, Fla.

NM 001 019 3 27 Dec. '48 Auricular Fibrillation Resulting from Hypothermia - A Case Report

NM 001 036 2 29 Dec. '48 A Spectrophotometric Method for the Continuous Quantitative Analysis of Nitrogen in Gas Mixtures

Naval Medical Research Unit No. 4, U.S. Naval Training Center, Great Lakes, Ill.

<u>Project</u>	<u>Report No.</u>	<u>Date</u>	<u>Title</u>
NM 007 023	Final	9 Dec. '48	Further Observations of an Inhibitor in Human Serums of the Hyaluronidase Produced by a Strain of Hemolytic Streptococcus

U.S. Navy Experimental Diving Unit, U.S. Naval Gun Factory, Washington, D.C.

SRD NO 1162/47	1	Sept. '48	Tests and Evaluation of Various Types of Oxygen Breathing Equipment for Use in the Recompression Chamber
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Note: Those interested in seeing copies of the complete reports should address their request to the research activity from which the report originates.

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Bone Banks to be Set Up in Naval Hospitals: The Bureau of Medicine and Surgery plans to set up bone banks in some of its naval hospitals. A study is being made to determine the particular naval hospitals in which such banks will be organized.

Through the use of bone banks, one operation may suffice instead of the two or more operations required when it is necessary to move bone from one area of the body to another, and, in the average case, operating time is shortened about 30 percent. Through the lesser amount of operating required in cases in which bone grafts are supplied from a bone bank, patients suffer less pain, which means requiring less pain-allaying drugs, and, having been spared a more extensive and longer procedure, recover more quickly. The employment of bone banks reduces the need for second operating teams for removal of the autogenous grafts. The routine practice of employing blood transfusions in bone-grafting operations is almost eliminated as a result of the more simplified operation in each case.

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Training Program for Naval Reserve Entomologists and Malariology Technicians: The Bureau of Medicine and Surgery has received many favorable comments concerning the training course for entomologists and malariology technicians at the U. S. Naval Air Station, Jacksonville, Florida.

The course is of two (2) weeks' duration. Training periods are scheduled to begin on the first and third Wednesday of each month, but if applicants for training find these dates cannot be met, other dates may be arranged inasmuch as no regularly scheduled classes are convened.

It is planned to continue this course during fiscal year 1950. Officers and men classified as entomologists and malariology technicians who are interested should apply by letter request to the Commanding Officer, Naval Air Station, Jacksonville, Florida, via the commandant of the naval district in which they reside. (Reserve Div., BuMed)

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Accidental Deaths in Naval Industrial Establishments, 1949: In the first two and one half months of 1949 there have been reported 11 deaths due to accidents among civilian employees in industrial establishments. This is exclusive of five deaths that occurred in a single plane crash at an ordnance test station which have not yet been determined as chargeable to Industrial Accidents. Eleven such deaths in less than 3 months is half of the number of accidental deaths reported for the entire year 1948. Because of this, consideration should be given toward the possible improvement of safety programs for example,

excellent opportunities exist during staff meetings and conferences for medical officers to point out this increase in the fatal accident rate and remind those more directly responsible of the need for more work on accident prevention. (Preventive Medicine Division, BuMed)

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Special Course for CO's of Volunteer Reserve Dental Units: A special course for commanding officers of Volunteer Reserve Dental Units will begin at the Naval Dental School, NNMC, Bethesda, Maryland on 25 April and will be of 14 days duration. The Bureau of Medicine and Surgery desires that each Volunteer Reserve Dental Unit throughout the United States be represented by the commanding officer of the unit, but if he be not available, by the executive officer or the dental officer next in seniority provided that he holds the rank of lieutenant commander or above.

The curriculum for the first week will cover the medical aspects of special weapons and radio-active isotopes and will be given in conjunction with a group of Naval Reserve medical officers. During the second week the Naval Reserve dental officers will meet independently, this time will be devoted exclusively to the problems of administration of Volunteer Reserve Dental Units and Dental Departments. Orders for those officers who will attend are being issued by the Commandants of the various districts.

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Courses in the use of Radioisotopes in Research: The Special Training Division of the Oak Ridge Institute of Nuclear Studies announces that courses entitled "The Technics of Using Radioisotopes in Research" are planned for the following periods:

- a. 6 June to 1 July, 1949 Inclusive
- b. 11 July to 5 August, 1949 Inclusive
- c. 8 August to 2 September, 1949 Inclusive

Each course will be divided into laboratory work, lectures on laboratory specialties, general background lectures, and special topic seminars. In order to give participants a maximum of knowledge and ability in the handling of radioactive isotopes in research, experiments will be conducted covering the use and calibration of instruments and the purification and separation of radioactive materials from inert materials and from other radioactive materials. Other laboratory periods will be devoted to the application of various radioisotope technics. Ample time will be allowed for library work.

Eight background lectures in nuclear physics are included in each course. A series of special-topic seminars conducted by leaders in the radioisotope field is an integral part of the course. Typical seminar topics are:

- Use of Radioisotopes in Animal Experimentation
- Use of Radioisotopes in Plant Experimentation
- Use of Radioisotopes in Humans
- Principles and Practice of Health-Physics in Radioisotope Work
- Design of Radiochemical Laboratories

Scope of the Use of Radioisotope Technics
Instrumentation for Radioisotope Work
Dosimetry

Participants, in cases in which it applies, are requested to bring with them plans of their laboratories, including layout, dimensions, equipment and similar information. The radioisotope training staff will be available to them for consultation in connection with efficient planning of laboratories for radioisotope work.

The course will be held in the laboratories and classrooms of the Oak Ridge Institute Building at Oak Ridge. No classified material is handled in the course, and the normal clearance procedure is not required. The Institute will arrange passes to admit participants to Oak Ridge.

Applications are desired from medical officers of the regular Navy for any one of the courses listed. Thirty-two (32) trainees will be accepted for each of the sessions. They will be selected from various sources and no quota has been assigned to the Navy. It is therefore desirable that applications be submitted at the earliest possible date; they must be received in the Professional Division, BuMed prior to 15 May 1949 to receive consideration. This type of training will be most advantageous for physicians serving in clinical and research assignments and they will receive preference when the final selection is made. It is requested that applicants include in their request a resumé of their professional qualifications and their reason for making application. Authorization orders, only, will be provided for attending these courses. No reliefs will be provided for medical officers authorized to attend. The entrance fee will be borne by BuMed. Applications are to be submitted to the Bureau of Medicine and Surgery via official channels and may be made by dispatch when necessary. (Professional Division, BuMed)

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ALNAV 27

21 March 1949

Subj: Re Program for Transfer to Regular Navy

ALNav 238-47 as modified by ALNav 52-48 is hereby superseded. The transfer program has been terminated except for those applying for appointment in Medical, Dental, Medical Service, or Nurse Corps.

Applications for appointment in Medical Service Corps limited to those from individuals who are graduates of accredited schools of pharmacy or optometry and those possessing a degree in a science allied to medicine. The Medical Service Corps portion of the transfer program will terminate on 1 May 1949, therefore all requests for transfer as Medical Service Corps officers must be submitted and in the mail on or before 1 May 1949. --SecNav

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BUMED CIRCULAR LETTER 49-33

23 March 1949

To: U. S. Naval Hospitals (except Houston and Aiea Heights) and Hospital Ships

Subj: Standard Clinical Record Forms: Forwarding of

Encl: 1. (HW) Series of Subject Forms

1. The Interagency Committee on Medical Records has developed a series of basic clinical record forms for use in all Federal hospitals, clinics, dispensaries, and similar facilities operated by the Federal Government. It is intended that these forms be utilized where pertinent, and that the series be increased later to include forms in a special request and treatment category such as obstetrics and gynecology.
2. The Bureau of the Budget has established the mandatory date for use of these forms as 1 January 1951. This will enable the Bureau to test the standard forms and develop procedures at selected naval hospitals prior to their general installation.
3. Enclosure 1 is forwarded for information only, since subject forms are not to be requisitioned until further instructions are issued by the Bureau. As additional forms are completed the Bureau will forward sample copies.

--BuMed. C. A. Swanson

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BUMED CIRCULAR LETTER 49-34

23 March 1949

To: All Holders of the Manual of the Medical Department

Subj: Advance Change 3-11, MMD

Encl: 1. (HW) Subject Change

1. The enclosed Advance Change 3-11 is effective immediately. It shall be recorded on the "Record of Changes" page in the Manual. The individual paragraph changes are to be inserted in their proper places in the Manual text.

--BuMed. C. A. Swanson

Note: This change will be distributed in about 3 or 4 weeks from the date of this letter.

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BUMED CIRCULAR LETTER 49-35

30 March 1949

To: All District and Staff Medical Officers

Subj: Conduct of Hospital Corps Professional Technical Examinations for Advancement in Rating

Ref: (a) Manual of Qualifications for Advancement in Rating (NAVPERS 18068)

1. Information has come to the attention of the Bureau that difficulty is being experienced by district and staff medical officers in coordinating Hospital Corps medical technical examinations in naval district and fleet-wide Hospital Corps competitive advancement examinations.

2. In order to clarify existing instructions and assure that Hospital Corpsmen designated in medical technical specialties are given opportunity to demonstrate specialized professional technical knowledge and skills, it is the desire of the Bureau that all men who so request be given either a practical, oral, or written examination in their designated specialty by the local examining board or medical officer conducting the examination, and an appropriate mark recorded on the final Examination Report or Answer Sheet.

3. It is further the desire of the Bureau that the examination mark in the technical specialty be incorporated as a factor in computing the final mark for the professional examination, in accordance with the instructions contained in the applicable sections of the "Manual of Qualifications for Advancement in Rating".

--BuMed C. A. Swanson

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BUMED CIRCULAR LETTER 49-36

30 March 1949

To: All Ships and Stations assigned Nurse Corps Officers

Subj: Interim Plan for Professional Examination for Promotion of Officers of the Nurse Corps

Ref: (a) BuPers C/L 18-49 of Navy Dept. Bull. dtd 31 Jan 1949, 49-63, (Paragraphs 3,4, and 10)

Encl: (A) Bibliography for promotion examination

This letter contains an interim plan to meet the present necessity of determining the professional qualifications of officers of the Nurse Corps eligible for promotion during the calendar year of 1949. The enclosure contains references to the material upon which the examining officers will base the examination. It is stated (1) that the nursing reference books listed were published as a result of a survey of the nursing field and indicate the present and future trends in the nursing profession, and (2) that all nurses should be acquainted with these books. --BuMed H. L. Pugh

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BUREAU OF MEDICINE AND SURGERY
WASHINGTON 25, D. C.

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